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AGRONOMY AND SOILS

Effect of Sample Size on Cotton Plant Mapping Analysis and Results

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ABSTRACT

Although cotton plant mapping has been valuable in understanding growth and development, variation in fruit distribution among plants is a significant mapping challenge. Choosing a sample that is large enough to generate useful information, but small enough to minimize time and resources, can make plant mapping more accessible for evaluating cotton crop growth characteristics throughout the cotton belt. The purpose of this research was to identify the effects of sample size and main-stem node grouping on sample variability. Plants were sampled in 10-m sections from six cotton cultivars at five locations in Georgia in 2009 and one location in 2010. The relative errors associated with sample sizes of one to 50 plants, as well as the statistical power associated with each sample size, were computed. On average, 37 plants per cultivar among five cultivars were required to reach a statistical power of 0.90, with the required number based on the magnitude of difference between cultivars in the fraction of plants having a boll at a given fruiting site. Grouping of main-stem nodes and the use of moving weighted averages decreased the error on a node-by-node basis. The use of these methods resulted in the loss of some node-by-node information that might be of value in particular cases, but the number of plants required to generate the same statistical power and standard deviation was decreased from a mean of 37 to a mean of 19 plants. These techniques should allow the use of smaller plant samples and make plant mapping more accessible.

The production and retention of cotton fruit throughout the plant canopy varies with cultivar and is sensitive to management, environmental conditions, and biological influences (Kerby et al., 2010). Planting mapping of fruit locations by main-stem node and sympodial fruiting position within the plant canopy is an important part of cotton research. The understanding of fruiting distribution has allowed researchers to decipher data observed in cotton yields and fiber quality more adequately.

Cotton fruiting occurs over a period of several weeks, with flowering occurring on vertical main-stem nodes up the plant on a 2- to 3-d interval and on adjacent fruiting positions on the same node on a 3- to 5-d interval (Bednarz and Nichols, 2005). Mapping might take several forms, but often consists of counting the cotton fruit at each fruiting site: the individual node and fruiting position of each fruit on the plant.

Fruit on different parts of the plant are at different maturity stages during times of periodic stress, insect pressure, or other factors that might affect growth. Because most of the plants within a cohort are at similar growth stages at a given time, fruit production and shedding can follow identifiable patterns among plants in a sample. End-of-season monitoring can be linked to crop history to show where and when the plants produced most of their crop, as well as what might have negatively impacted yield (Kerby et al., 2010).

Cotton has been shown to have different fruit development and distribution patterns based on several factors, including cultivar, plant density, and plant growth regulator (PGR) application (Bednarz et al., 2000; Dumka, 2002; Dumka et al., 2004). Moisture deficit has also been shown to affect boll distribution, as shown by Pettigrew (Pettigrew, 2004a, 2004b) and Ritchie et al. (2009). Differences in yield distribution and fiber quality have been observed based on cultivar and genetic technology (Bauer et al., 2009; Mills et al., 2008). All of these findings have helped expand the knowledge of cotton growth and developmental habits.

Current Plant Mapping Methods. Plant mapping can take the form of either in-season measurements of the production and growth of fruit (Bednarz and Nichols, 2005; McClelland, 1916), or end-of-the season measurements of boll yield components, such as node-by-node boll fraction (the fraction of plants with a boll at a given fruiting site), yield, percent lint,

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and fiber content and quality (Bauer et al., 2009; Bednarz and Nichols, 2005; Pettigrew, 2004b). Bauer et al. (2009) and Bednarz et al. (2006) both identified differences in fiber quality parameters within different regions of the plant.

One question that has arisen regarding the use of plant mapping is the number of plants required to provide adequate statistical power to separate differences in treatments while using labor and time resources efficiently. Within a representative sample composed of multiple plants, some nodes and fruiting positions exhibit higher or lower fractions of fruit, but it is unusual for every plant within a sample to consistently have fruit on any single fruiting site.

Furthermore, competition between plants, as well as between fruiting sites on an individual plant, can affect boll distribution (Boquet and Moser, 2003; Kerby and Buxton, 1981; Pettigrew, 1994). Plant mapping in any form is a time-consuming process, and additional measurements such as boll mass and lint percentage can limit the scope of the process to only a relatively few samples per year. Researchers have tried varying sample sizes, including plant areas such as 0.5 to 1.0 m^2 of row (Sadras et al., 1997), 1 m^2 of row comprising about seven plants (Constable, 1991), row lengths of 1 m (Pettigrew, 2004b), 2 m (Cook and Kennedy, 2000), and 3 m (Bednarz and Roberts, 2001; Bednarz and Nichols, 2005; Mills et al., 2008) to plant counts of 10 (Vories and Glover, 2006) or 20 plants (Boquet and Moser, 2003; Boquet et al., 1994). The variation in sample size between studies shows the balance that researchers try to make between having an appropriately large sample size and limiting the numbers of plants in a sample to save time or allow additional plots to be measured.

Statistical Power and Plant Mapping. The use of statistical power has been used successfully in biological research to determine the associated risks of overlooking real differences between treatments due to insufficient sample size (Thomas and Juanes, 1996). Statistical power is the probability of a significant population difference resulting in a statistically significant sample difference, and measures the sample size required to maintain a significant result if the null hypothesis is false. Specifically, a test of statistical power estimates the Type II error: the risk of a real result being masked by a test that is not sensitive enough to detect treatment differences. An analysis of the statistical power of different subsample row lengths should give insight on the proper subsample size to minimize the risk of overlooking significant differences while limiting the number of plants that must be sampled.

Data Smoothing. In addition to determining variability and statistical probability on a node-by-node basis, it might be possible to decrease variability and therefore improve statistical power through the use of node grouping or smoothing techniques (Savitzky and Golay, 1964). Grouping nodes together decreases the effects of node-to-node variability in the sample by taking into account adjacent nodes. However, the grouping of nodes limits information to broad areas of the plant. This method is likely appropriate in experiments resulting in small treatment effects on boll distribution where differences between individual nodes are subtle, but overall differences within a region of the plant may be significant.

Smoothing can also be used as a method to decrease variability, and is also widely used as a method of statistical "noise" removal in many facets of science, including spectrometry (Demetriades-Shah et al., 1990; Elvidge and Chen, 1995), speech recognition (Kulkarni and Colburn, 1998), and time series analysis (Chen et al., 2004). A smoothing function uses values from adjacent data points to decrease point-to-point variation over a series. The purpose of smoothing is to remove small scale variations and noise from a spectrum or time series, while preserving most of the useful features. In the case of boll distribution measurements, decreasing node-to-node variability with a smoothing function should significantly decrease the error, and therefore, the number of plants that would be needed for a strong analysis.

Our objectives in this study were to measure the relative error associated with different subsample populations within a plot to give more clarity to the plant mapping process and test methods of decreasing the associated error without increasing the number of plants being sampled. With this additional information, the use of plant mapping techniques can be expanded without increasing the time required.

MATERIALS AND METHODS

Nonirrigated county variety trial locations in Burke, Colquitt, Coffee, and Jefferson counties in Georgia, and an irrigated trial in Coffee County were used as locations for data collection in 2009. The trials were conducted under the direction of county extension agents and extension specialists throughout South Georgia and East Georgia. At each location, 10 m of linear row were selected in each of six cultivars: Delta & Pineland 555 BR (DP555), Delta & Pineland 0949 B2RF (DP0949), Delta & Pineland 0935 B2RF

(DP0935), Stoneville 5458 B2RF (ST5458), FiberMax 1740 B2RF (FM1740), and Phytogen 375 WRF (PHY375). These cultivars were chosen for their widespread use in Georgia and their unique fruiting habits.

Data Collection. Plant heights and spaces between plants were measured for each individual plant within the 10 m of row, and all plants were mapped that were at least 45 cm tall and had at least one viable fruit. Plant mapping consisted of counting all harvestable fruit by fruiting site on both vegetative and reproductive branches on each individual plant. Bolls produced by vegetative branches were summed across the entire plant and the main-stem node and sympodial fruiting position were recorded for each boll produced on a fruiting branch. Total main-stem nodes for each plant were also measured. Plant density ranged from five to eight plants per square meter and varied by location and cultivar. Data were collected between 2 and 10 d prior to harvest, and at least 1 wk after defoliation. Harvest data included plot length and seed cotton weight for the entire plot, and 100 kg subsamples were ginned at the University of Georgia Microgin.

Analysis. After mapping was completed, the effects of sample size were analyzed by grouping every possible range of 1 m, 1.5 m, 2 m, and 3 m of plants in a linear row as individual samples and measuring the relative error from the means of each grouping size for each node and fruiting position.

The effects of using basic selection procedures to determine fruit distribution on a plant-by-plant basis were also tested with samples of 5, 10, 15, and 20 plants. Plants with apical meristem damage below node 15 that caused loss of apical dominance, plants that were more than one standard deviation shorter than the cultivar mean at each location, and individual plants with a cumulative gap between adjacent plants of greater than 40 cm were eliminated from the analysis. This method simulated basic decision making in a trial situation to eliminate nonrepresentative plants and potentially give a more precise estimate without an increase in the amount of sampling. Because the purpose of the study was to determine the relative uncertainty of different sample sizes, the standard deviation generated with each sample size and method was used as a basis for measuring relative error.

The error ratio was calculated by measuring the standard deviation at all existing plant boll populations for each sample size ($s_{n\ plants}$), regressing the error to the error for one-plant samples ($s_{1\ plant}$), forcing the intercept to 0, and measuring the slope, or ratio, as $s_{n\ plants} / s_{1\ plant}$. This allowed comparison over the entire range of boll fractions from the samples, even though the standard deviation varied based on boll fraction between nodes and cultivars. The same calculation was performed with smoothed data at the same plant sample sizes, and the error ratio was calculated based on the unsmoothed $s_{1\ plant}$ to show the relative decrease in error compared to unsmoothed data.

Power analysis was also performed in SAS 9.2 (SAS Institute, Inc., Cary, NC) using PROC POWER to measure the number of samples necessary to have a reasonable certainty (statistical power of 0.9) of differences by node between cultivars in the five environments. The standard deviations of the full 10-m plot lengths were used as the standard deviation for the power analysis.

RESULTS AND DISCUSSION

Plant Number and Relative Error. Standard deviation was closely related to the percentage of plants with bolls at a specific node and position (Fig. 1a) over a wide range of sample sizes. Counting bolls on a plant-by-plant basis results in a binary dataset: each plant either has or does not have a boll at a specific node and position.

As shown in Fig. 1a, the relationship between measured standard deviations and boll fraction followed a very predictable model. The standard deviation lines by sample size in Fig. 1a were derived from a 10,000-sample binary dataset, but none of the standard deviation measurements by location varied from the single-plant standard deviation line, even though bolls were not necessarily randomly distributed among the plants in the samples. Grouping plants together resulted in variations in the standard deviation by boll fraction, due to the increase in the sample size compared to the population size, the decrease in the number of samples, and the effects of nonrandom distribution of bolls within the population (Fig. 1b).

The accumulation of additional plants within a sample approximates a continuous dataset as sample population increases. At high and low proportions of plants with fruit at a given node and position, the standard deviations were low even at low sample populations, because there were fewer samples that varied from the mean. However, as the ratio of plants with a boll approached 0.5, the standard deviation within a group of individual plants increased to almost 0.5. Increasing sample size decreased the standard deviation significantly (Fig. 1a); an increase from one plant to five plants in a sample decreased the overall standard deviation by 56% (Fig. 2).

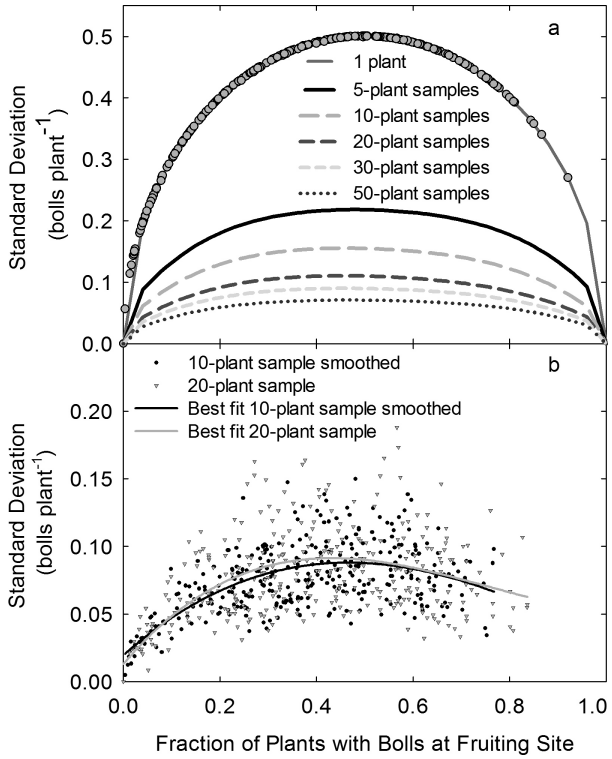

Figure 1. Relationship between fraction of plants with bolls and standard deviation (a) for five sample sizes with a random distribution of plants with and without bolls and (b) with 10-plant samples with data smoothing and 20-plant samples without data smoothing.

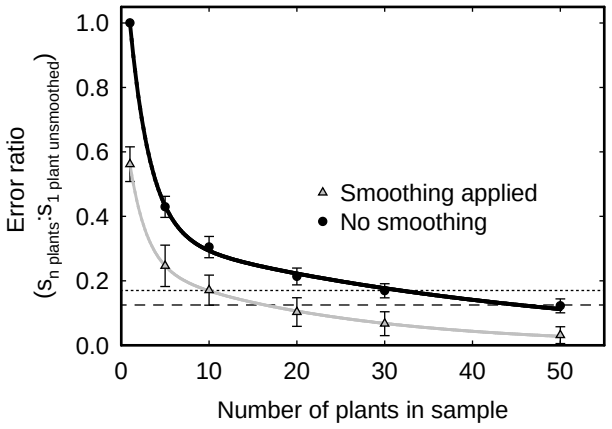

Figure 2. Relative standard deviation ($s_{n\ plants}$ / $s_{1\ plant\ unsmoothed}$) based on the number of plants in a plant mapping sample compared to that of a single plant. The black line represents measurements taken from individual nodes with no smoothing. The gray line represents a five-node weighted average moving window smoothing function. Error bars represent the standard error of the individual $s_{n\ plants}$ / $s_{1\ plant\ unsmoothed}$ ratios for each number of plants. The horizontal lines indicate the relative error at 30 and 50 plants for the unsmoothed data.

The decrease in the $s_{1\ plant}$ / $s_{n\ plants}$ ratio with the addition of plants was nonlinear, becoming less pronounced as plant number increased (Fig. 2). Measuring five plants instead of one decreased the standard deviation by 56%, and the use of 10 plants decreased the standard deviation by 70%. The addition of plants into a sample greater than 10 plants had increasingly smaller effects on standard deviation: 20 plants decreased the standard deviation by 79%, 50 plants decreased it by 87%, and 100 plants by 91%.

Power analysis for cultivar separation for first position fruit at each node showed the number of samples required for a statistical power of 0.9 (10% or less Type II error) to range from 9 to 141 with a mean of 41-plant samples and a median of 32-plant samples (Table 1). Nodes and locations with smaller ranges of differences between cultivars required higher numbers of samples to reach a statistical power of 0.9, with particularly high numbers occurring when all pairwise differences in the boll fraction between cultivars at a specific node were less than 0.07.

The standard deviation versus sample size comparisons for both plot-length measurements and plant-number measurements resulted in nearly identical relationships, even though stunted plants, damaged plants, and plants with large gaps around them were removed from the plant-number measurements and not from plot-length measurements (Fig. 3). However, the seeming lack of effect by removing nonstandard plants might be misleading. Standard deviation is measured from the population at large, and changes in fruiting at a given fruiting site for a single plant affect both the overall fruit proportion and the associated standard deviation. Measurement of standard deviation does not directly take into account the effects on fruit proportion, so nonstandard plants have no different effect on boll fraction at a specific fruiting site than any other plant. However, mapping unusual plants can increase or decrease the measured boll fraction outside of the natural range among typical plants.

Grouping of Adjacent Nodes and Smoothing. The grouping of adjacent nodes was shown to decrease measurement error, as measured by standard deviation, and decrease the number of plants required to reach a given standard deviation (Fig. 4). Combining groups of two nodes resulted in a small decrease in error for all measurement lengths, and combining groups of four nodes decreased measurement error substantially at all measurement lengths. However, some of the ability to assess growth habits was lost by combining additional nodes into zones. For the four-node groupings, measurements of unique values were decreased from 19 to 5.

Table 1. Power analysis for cultivar mean separation at all locations. Sample size represents the plant sample size required to obtain a statistical power of 0.9 for first position boll differences between cultivars

	No Smoothing							Weighted Smoothing					
	Environment							Environment					
node	1[z]	2	3	4	5	Mean	node	1	2	3	4	5	Mean
	Required Sample Size							Required Sample Size					
7	32	81	22	17	30	36	7	16	20	12	11	15	15
8	13	34	17	20	38	24	8	10	11	9	9	13	10
9	17	27	15	22	20	20	9	8	8	9	9	13	9
10	17	21	32	25	49	29	10	8	7	13	11	28	13
11	23	18	34	38	141	51	11	11	8	18	14	90	28
12	28	26	61	59	31	41	12	20	11	30	19	28	22
13	80	62	38	41	60	56	13	55	27	30	20	22	31
14	32	104	69	63	57	65	14	18	55	22	24	21	28
15	19	54	57	92	85	61	15	10	29	18	70	25	30
16	17	24	40	114	54	50	16	8	16	15	82	24	29
17	22	18	39	52	82	43	17	8	11	14	33	27	19
18	19	18	23	63	87	42	18	7	11	13	27	35	19
19	13	20	27	47		27	19	7	12	15	20		14
20	9	31	38	17		24	20	7	15	20	11		13
21	32	81	22	20		39	21	16	20	12	9		14
Mean	25	41	36	46	61	41	Mean	14	17	17	25	28	20

[z] Environments were Burke (1), Colquitt (2), Coffee Dryland (3), Coffee Irrigated (4), and Jefferson (5)

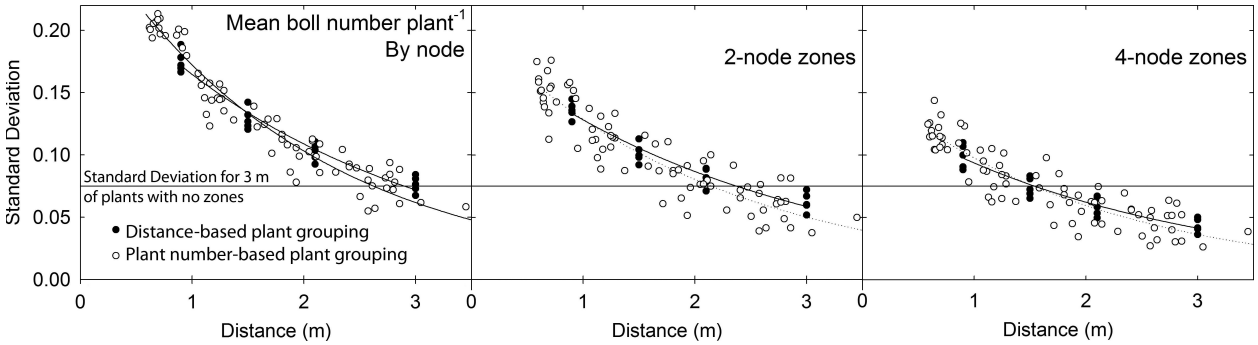

Figure 3. Mean boll number per plant standard deviation versus measurement distance by first position node with no zones, two-node zones, and four-node zones. A reference line is added for comparison of the relative error of each zone method compared to a 3-m sample with no zones.

The use of a moving average was tested and found to decrease standard deviation while maintaining some of the node-to-node variability (Fig. 4). The characteristics of a moving average with both a numerical mean (equivalent to grouping adjacent nodes with a moving window for each node) and a weighted mean were found to have similar effects on the data, but with slight differences. The numerical means resulted in slightly lower standard deviations on a node-by-node basis, but the deviations from the mean of the unsmoothed data were larger than with the weighted mean method (data not shown), suggesting an increased loss of resolution due to the numerical mean. Because the differences between the numerical mean and the weighted mean were small, the weighted mean was used for additional analysis to decrease variability while minimizing variations from the unsmoothed data. Using a weighted mean decreased the number of plants required per sample to give a significant result between cultivars (Table 1), generally decreasing the sample size requirements in half.

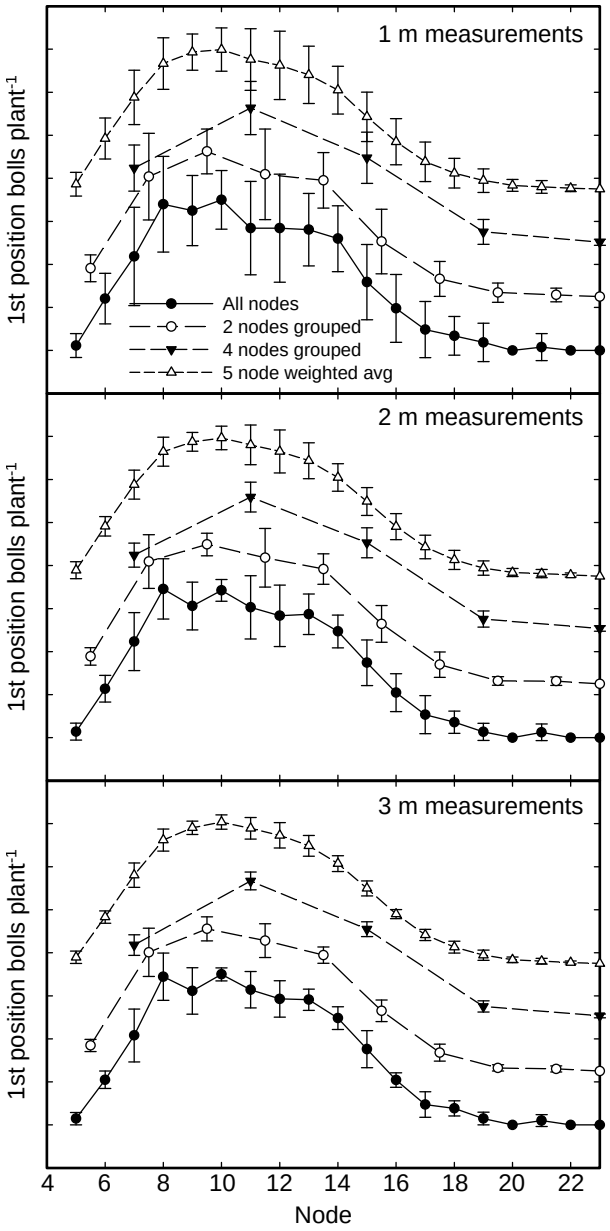

Figure 4. Means and standard deviations at individual nodes, with two- and four-node groupings, and with a five-node weighted average for all nodes at 1 m, 2 m, and 3 m spacing using data from the DP0935 plot at location 2. For node groupings, values are calculated at the midpoint of each grouping. Ticks represent 0.2 fruit per plant, and node groupings are offset by 0.25 to allow comparisons.

The use of the five-node smoothing procedure resulted in lower numbers of plants required to reach low $s_{1\ plant} / s_{n\ plants}$ ratios (Fig. 2). The $s_{1\ plant} / s_{n\ plants}$ ratio of a group of 10-plant samples with smoothed data was almost identical to the error ratio obtained with a group of 30-plant samples and no smoothing. The $s_{1\ plant} / s_{n\ plants}$ ratio with 50-plant samples and no smoothing could be duplicated with 17-plant samples and smoothing. Similarly, the use of a 10-plant sample with smoothing resulted in an almost identical standard deviation by boll fraction as the use of a 20-plant sample with no smoothing (Fig. 1b).

The effects of smoothing on different plant sample sizes are shown in Fig. 5. For a single plant, the acute differences between present and absent bolls were muted by the smoothing, but the distribution did not resemble the overall boll distribution. However, with five plants in a sample, the smoothed data already resembled the data with 50 plants in a sample. Most of the plants at the beginning of the measurement row did not have bolls at node 12, whereas most of the plants near the end of the row did have bolls at node 12. Therefore, lower populations had significant deviations from the 50-plant sample, whether smoothing was applied or not. However, the differences were spread out over a broader node region when the data were smoothed, so the differences were less apparent.

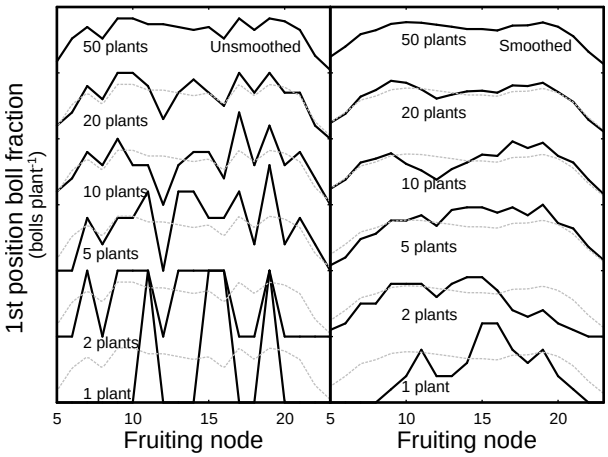

Figure 5. Effects of smoothing on first position boll distribution in sample sizes ranging from 1 to 50 plants. Boll fraction data are offset by 0.5 to allow comparisons. The dotted grey line represents the 50-plant sample for the corresponding method.

In research where heavy emphasis is placed on boll fraction at a specific fruiting site, smoothing the data might hide some of the effects. However, when overall distribution is of interest, smoothing results in cleaner data. As shown in Fig. 6, use of a weighted average can make overall boll fraction trends much more distinct, both for first- and second-position fruit. Boll fraction trends between nodes and differences between cultivars were visually apparent even in cases where there was overlapping boll distribution between cultivars.

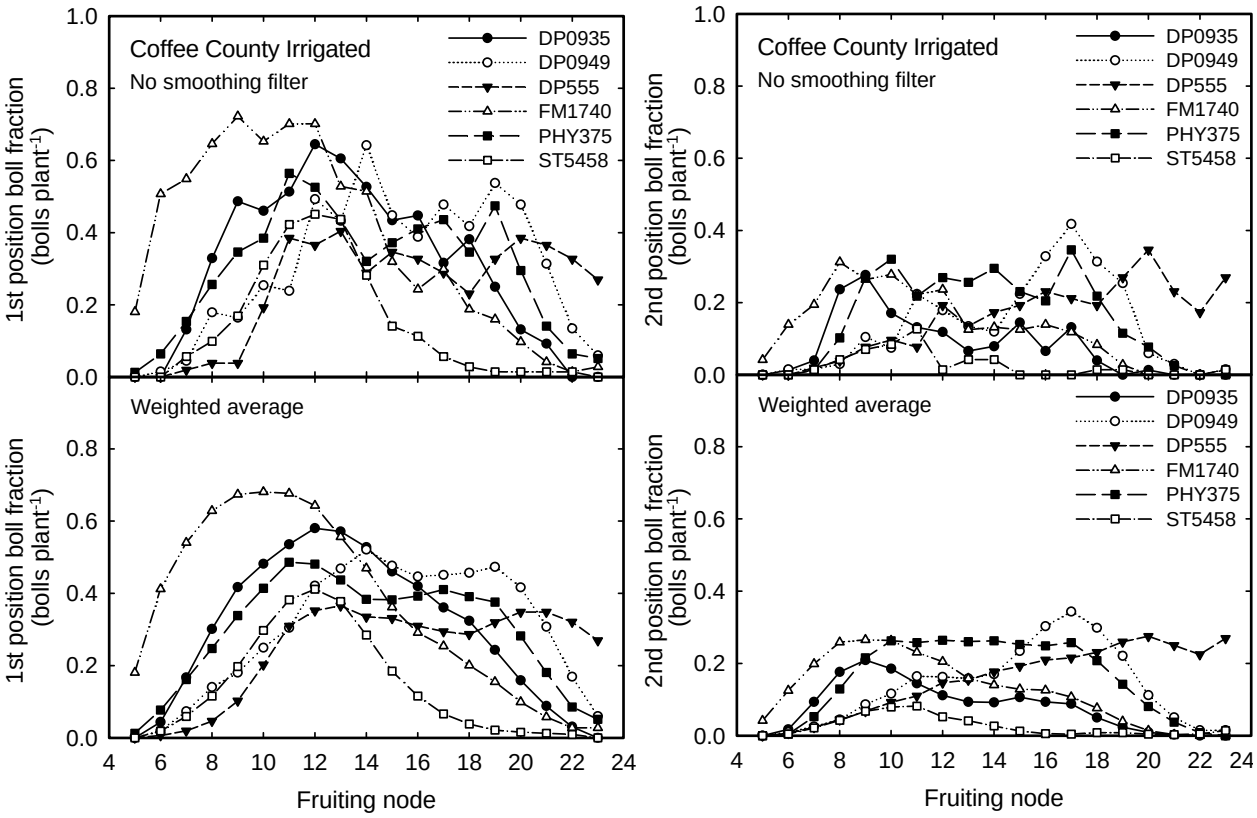

Figure 6. First position and second position boll fraction cultivar comparisons without filtering and with weighted average filtering.

Sequential Sampling and Plant Smoothing. One suggested method of decreasing the amount of time measuring samples is the use of sequential sampling methods. Sequential sampling methods have been used for more than 50 y in insect scouting (Waters, 1955; Morris 1960), and are based on the concept that when there are either high or low populations of an item of interest, fewer samples are required to accurately estimate the population. A similar level of confidence can be obtained from a few plants if nearly every plant has a boll present at a given fruiting site, as with a larger sample where the plants are evenly divided between having and not having a boll at a given fruiting site.

Counting bolls would certainly follow this conceptual model, but there are at least three factors that should be considered when using this method in plant mapping. These factors include whether more than one fruiting site is of interest in the study, the effects of adjacent fruiting sites on the site of interest, and whether smoothing will be applied.

Unless only one fruiting site is of interest in the study, each fruiting site encountered will have a different boll complement across a sample of multiple plants. Therefore, a simple measure of the number of plants required for a measurement is complicated by the differences between boll fractions at different fruiting sites. Because many of the differences in fruiting distribution between plants occur on parts of the plant where fruit retention is not high, there is a risk of choosing too few plants to ascertain differences if sampling is limited by the number of plants measured due to high boll retention at another part of the plant.

The second consideration is the effect of adjacent fruiting sites on boll distribution. Kerby and Buxton (1981) determined that when a fruit is lost, adjacent fruiting sites are more likely to retain bolls. Plant-to-plant variability can result in compensation on different fruiting sites, so a focus on an individual fruiting site will sometimes overlook the effects of adjacent sites. Using smoothing methods can reduce the effects of site-to-site variability, as well as take into account the effects of adjacent fruiting sites. However, smoothing also makes determination of the proper number of plants to measure difficult, because an estimate of the boll fraction is not tied completely to any single fruiting site.

CONCLUSIONS

The question of how many plants to sample for an accurate representation of boll distribution does not have a simple answer, because variance at each fruiting site is directly related to boll fraction, and each fruiting site has its own boll fraction. Furthermore, the very factors that plant mapping measures, such as cultivar, irrigation, and chemical application effects, influence growth habits and make prior determination of treatment effects difficult over a range of multiple fruiting sites. Because many of the differences due to these factors are observed in regions of the plant with low-to-medium boll fractions, it is advisable to determine the number of plants required based on the highest standard deviation for a sample with a given number of plants. However, the use of smoothing can decrease the number of samples required to reach a set standard deviation, regardless of the initial boll fraction.

In our research, increasing the number of plants in a sample decreased the standard deviation, with sample sizes of 10 plants or more resulting in standard deviations that became marginally lower with the addition of each additional plant. Grouping nodes or subjecting the data to a moving average decreased variance within a sample, in most cases decreasing the number of plants required to reach a critical statistical power by half. Although sample sizes of 40 plants were necessary in many cases to reach a statistical power of 0.9 without moving averages, the use of multiple replicates can be used to reach this number in cases where plant growth is similar between replicates.

There is a risk with moving averages that some of the underlying individual node data might be lost, particularly in cases where outside factors might affect fruit production or retention for a short period of time. Some outside factors that can affect fruit retention over a short period of time include insect damage, periodic drought stress, and periods of temperature extremes (Bednarz and Roberts, 2001; Bednarz and Nichols, 2005; Burke, 2003). Therefore, different trends might be observed in analysis by individual node than are observed when the nodes are subjected to smoothing.

Yield distribution measurements offer the potential of significant insights into cotton development and yield potential, which might have been overlooked due to the time required to perform mapping and the complexity of analyzing a crop that has multiple nodes and fruiting positions concurrently producing and shedding fruit. Smoothing might give additional insights into interactions between fruiting positions by decreasing node-by-node variation and clarifying patterns that might otherwise be overshadowed by noise.

A suitable sample size will depend upon both the overall boll fraction and upon variability within a sample. Additionally, variability between replicates can affect necessary sample size. However, in cases where boll distribution is significantly different between treatments, a sample of 30 to 40 plants should be enough to see this difference. If boll distribution does not differ substantially between replicates, sample sizes that result in the sampling of 8 to 10 plants within a replicate should suffice. Using smoothing functions can further decrease sample size, with the required sample size being decreased by half in our testing.

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